

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :  A61K 7/48		A1	(11) International Publication Number: WO 94/20068  (43) International Publication Date: 15 September 1994 (15.09.94)
(21) International Application Number: PCT/US93/02113  (22) International Filing Date: 10 March 1993 (10.03.93)		(81) Designated States: AT, AU, BB, BG, BR, CH, DE, DK, FI, GB, HU, JP, KP, KR, LK, LU, MG, MW, NL, NO, RO, RU, SD, SE, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).	
(71) Applicant: YALE UNIVERSITY [US/US]; Suite 401, 246 Church Street, New Haven, CT 06510 (US).		Published <i>With international search report.</i>	
(72) Inventors: PAWELEK, John, Mason; 154 Woodlawn Street, Hamden, CT 06517 (US). BOGNIA, Jean, Lynn; 140 Patten Road, North Haven, CT 06473 (US).		(74) Agent: BALDWIN, Geraldine, F.; Pennoe & Edmonds, 1155 Avenue of the Americas, New York, NY 10036-2711 (US).	
(54) Title: COMPOSITION AND METHOD FOR WHITENING SKIN			
(57) Abstract  A skin-whitening and suntan-inhibiting composition comprising (a) a compound which depletes glutathione, and (b) hydroquinone or an alkyl or aralkyl ether of hydroquinone.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Larvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

COMPOSITION AND METHOD FOR WHITENING SKIN

This invention was made with government support under grant number R-814125 awarded by the U. S. Environmental Protection Agency. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The invention relates to compositions and methods for the whitening of skin and hair.

U. S. Patent 4,990,330 discloses compositions for topical use having melanin synthesis-inhibiting activity comprising kojic acid or its esters and at least one compound selected from the group consisting of azelaic acid, tropolone, oipoic acid, sorbic acid, glucosamine, derivative of glucosamine, tunicamycin, deoxynojirimycin, glutathione, cysteine, hydroquinone, derivative of hydroquinone, dehydroacetic acid, chelidonic acid and lipoamide. Such compositions are disclosed as having excellent human skin-whitening and anti-suntan effects.

OBJECT OF THE INVENTION

It is an object of the invention to provide a novel, efficient, and safe way of whitening skin and hair in mammals and inhibiting the pigmenting effects of exposure to the sun.

Another object of the invention is to provide novel compositions to effect such whitening and inhibiting of sun-mediated pigmentation.

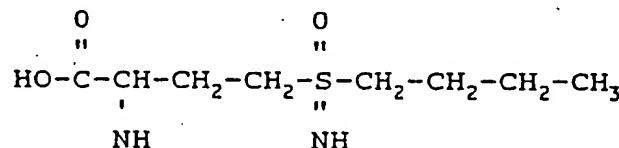
#### SUMMARY OF THE INVENTION

These and other objects are realized in accordance with the present invention pursuant to which there is administered to a mammal, preferably a human, an amount effective to achieve whitening of a compound which depletes glutathione, preferably a selective inhibitor of  $\alpha$ -glutamyl synthetase and more preferably buthionine sulfoximine.

Advantageously the material applied also contains hydroquinone or an alkyl or aralkyl ether of hydroquinone.

#### DETAILED DESCRIPTION OF THE INVENTION

Buthionine sulfoximine is a known compound of the formula



While it has been disclosed that it kills human melanoma cells grown in culture, it has not been disclosed that it has a whitening effect on human skin and hair.

In accordance with the invention, buthionine sulfoximine can be administered alone or preferably in combination with hydroquinone or an ether thereof, as

specified hereinabove; hydroquinone and ethers thereof are known to have a whitening effect. Surprisingly, however, the combination of sulfoximine with hydroquinone or an ether thereof exhibits more than an additive effect.

In such joint administration, the buthionine sulfoximine can be replaced by other compounds which deplete glutathione, particularly those which selectively inhibit  $\alpha$ -glutamyl synthetase.

The materials may be applied alone but are preferably administered dissolved or suspended in a carrier which may comprise water, conventional skin moisturizers and creams, and the like. Advantageously, such composition also contains an antioxidant, for which purpose ascorbic acid has been found especially useful.

The active materials can be administered in any manner but are preferably applied topically, e.g., by pouring or rubbing on the skin in the area sought to be whitened.

The selective inhibitor of  $\alpha$ -glutamyl synthetase, e.g. buthionine sulfoximine, is advantageously applied in a carrier wherein it is present in a concentration of about 0.025 to 0.5 mole per liter.

As noted, the  $\alpha$ -glutamyl synthetase inhibitor (a) is advantageously administered in conjunction with (b) hydroquinone or an alkyl or aralkyl ether thereof, e.g. the mono-benzyl ether.

When (b) is present, the weight ratio of (a):(b) ranges from about 10:1 to 1:10, advantageously from about 5:1 to 1:5 and preferably about 1:1.

When an antioxidant (c), such as ascorbic acid, is present desirably it is present in about 0.25 to 5% by weight, preferably about 1% by weight.

The invention will be further described in the following illustrative examples wherein all parts are by weight unless otherwise expressed.

Example 1

Mouse melanoma cells ( $4 \times 10^6$ ) were grown in conventional cultures.

Portions were treated with:

- i) nothing;
- ii)  $2 \times 10^{-7}$  M melanocyte stimulating hormone (MSH) plus  $10^{-4}$  M isobutylmethylxanthine (IBMX);
- iii) MSH and IBMX plus  $1 \times 10^{-6}$  hydroquinone (HQ);
- iv) MSH and IBMX plus  $5 \times 10^{-6}$  M buthionine sulfoximine (BSO);
- v) MSH and IBMX plus HQ and BSO.

Following treatment, cells were collected by centrifugation and photographed. Results were as follows:

(i) showed no color change and remained white; (ii) darkened considerably as expected, due to the known pigmenting effects of MSH and IBMX; (iii) and (iv) were slightly

lighter than (ii); (v) was as white as (i) even though MSH and IBMX were present. The conclusions are that BSO in combination with HQ elicits a strong whitening effect on pigment-producing cells, preventing the induction of pigment by hormones. A further conclusion is that since MSH is known to be a key intermediary in ultraviolet light-induced pigmentation, it is implied that BSO and HQ also elicit inhibition of sun-mediated pigmentation.

Example 2

Ears and surrounding hair-bearing skin of living C57 black mice were rubbed daily with an aqueous solution containing 25% by weight of glycerol, 0.1 molar TRIS (trihydroxyaminomethane), buffered to p.H 6.8, to which there was added (A) nothing, (B) 4% by weight of hydroquinone, (C) 5% by weight of buthionine sulfoximine, and (D) 4% by weight of hydroquinone plus 5% by weight of buthionine sulfoximine. After 15 days (A) and (C) showed no depigmentation of the surrounding hairs where rubbed, (B) showed noticeable depigmentation and (D) showed marked depigmentation.

Example 3

The lower backs of living C57 Black mice, from which hair had been removed prior to treatment, were rubbed daily with Acid Mantle Creme (Sandoz Pharmaceuticals Corp.)

containing aluminum sulfate, calcium acetate, cetearyl alcohol, glycerin, light mineral oil, methylparaben, purified water, sodium lauryl sulfate, synthetic beeswax, white petrolatum, white potato dextrin, ammonium hydroxide and citric acid, to which there was added (A) nothing (control), (B) 5% by weight buthionine sulfoximine (BSO), (C) 4% by weight hydroquinone (HQ), and (D) 5% by weight of buthionine sulfoximine plus 4% by weight of hydroquinone (BSO/HQ). Treatment was continued until new hair had grown out in the previously plucked regions (about 15 days). Approximately 100 new hairs from the treated areas were removed and scored under a microscope by two independent observers for the percentage of white hairs amongst the black hairs. (A) and (B) showed no white hairs, (C) showed about 2% white hairs, (D) showed about 14% white hairs. Thus BSO in combination with HQ is almost an order of magnitude more effective in eliciting whitening than either agent used alone.

#### EXAMPLE 4

Composition and Method for Eliciting Whitening of Human Skin and for Inhibiting the Pigmenting Effects of Ultraviolet Light

Acid Mantle Creme (Sandoz Pharmaceutical Corporation) (or other suitable carriers known to the art)

containing 1-4% by weight hydroquinone and 1-5% by weight buthionine sulfoximine.

Directions: Apply daily until the desired whitening or prevention of sun tanning is achieved.

It will be appreciated that the instant specification and claims are set forth by way of illustration and not limitation, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS

1. A skin-whitening and suntan-inhibiting composition comprising (a) a compound which depletes glutathione, and (b) hydroquinone or an alkyl or aralkyl ether of hydroquinone.
2. A composition according to claim 1, wherein (a) is a selective inhibitor of  $\alpha$ -glutamyl synthetase.
3. A composition according to claim 1, wherein (a) is buthionine sulfoximine.
4. A composition according to claim 1, wherein (b) is hydroquinone or hydroquinone mono-benzyl ether.
5. A composition according to claim 1, further comprising (c) an antioxidant.
6. A composition according to claim 5, wherein (c) is ascorbic acid.
7. A composition according to claim 5, further containing a carrier, (b) being present in about 0.025 to 0.5 mole per liter, the weight ratio of (a):(b) ranging from about 10:1 to 1:10, and (c) being present in about 0.25 to 5% by weight based on the composition.

8. A method of whitening skin which comprises applying to the skin an amount effective therefor of a composition according to claim 1.

9. A method of whitening skin which comprises applying to the skin an amount effective therefor of a composition according to claim 7.

10. A method of whitening skin which comprises applying to the skin an amount effective therefor of buthionine sulfoximine.

## INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/US 93/02113

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR,A,2 152 442 (E.M.E.R.A. SA) 27 April 1973 see the whole document ---	1-10
A	EP,A,0 269 017 (CETUS CORPORATION) 1 June 1988 see page 5, line 46 - line 52 see page 14, line 1 - page 16, line 50 see claims 1,4,7-10 ---	1-10
A	S.T.N., Serveur de Bases de Données, KARLSRUHE, DE, Fichier Chemical Abstracts, vol 115, n 109447, 1991 see the abstract -----	1-10

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

14 February 1994

25.02.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 cpo nl.  
Fax (+ 31-70) 340-3016

Authorized officer

Fischer, J.P.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/02113

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A-2152442	27-04-73	NONE		
EP-A-0269017	01-06-88	CA-A-	1321349	17-08-93
		DE-A-	3780770	03-09-92
		ES-T-	2042529	16-12-93
		JP-A-	63190831	08-08-88
		US-A-	4985241	15-01-91